Adhesion (Crystal Violet assay)

Warning: Crystal Violet is a suspected carcinogen. Crystal Violet will stain your skin or clothes by contact and cannot easily be removed. Use gloves at all times when handling Crystal Violet.

With this protocol you can compare adhesion ability/biofilm forming ability of isolated clones/mutants. The adhesion assay can be used as the first screening of a series of strains before investigating the most interesting ones in more details in our flow-chamber system.

Day 1:

Inoculate strains you want to analyze in 10 ml test tubes. Use dilute media to avoid excessive growth.

Day 2 (or when the plats have suitable growth):

Inoculate microtiter plate with the different strains of interest. Use 150 µl medium for each well. Use suitable micrtotiter plates. U-bottom or flat-bottom are suited. 0.1% Crystal Violet solution [0.1 g Crystal Violet in 100 ml water].

1. Use 2 microtiter plates for each set of clones
2. Place the inoculated microtiter plate in a thick plastic bag and incubate over night at 37ºC or 30C (depending on strain etc., also, some clinical isolates needs two/tree days' incubation).

Day 3:

3. After incubation empty all the wells by simply throwing out the liquid in a clinical waste bag (without using a pipette).
4. Transfer pre-warmed (37ºC or 30ºC) medium or 0.9% NaCl to a sterile Petri dish and use a multi-pipette to gently add 200 µl (avoid overloading) medium/NaCl to each well, and discard it by throwing. Repeat this washing step twice.
5. Add 200 µl (avoid overloading) Crystal Violet solution to each well (**use gloves**) and let stand on the table for 15-20 min.
6. Discard the Crystal Violet solution by throwing (**use gloves, beware of splashes**).
7. Wash tree times with water (3x200 µl (avoid overloading)). Again discard by throwing.
8. Add 200 µl (avoid overloading) 96% ethanol; pipette up and down thoroughly in order to dissolve the Crystal Violet (use multi-pipette).
9. Read the plate in an Elisa reader using an excitation of 585 nm and make a column diagram data using spreadsheet software.

Reference:

Reisner A, Krogfelt KA, Klein BM et al (2006) In vitro biofilm formation of commensal and pathogenic Escherichia coli strains: impact of environmental and genetic factors. J Bacteriol 188: 3572-3581